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Health and Environmental Effects Profile for 2-Methoxy-5-Nitroaniline

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR 2-METHOXY-5-NITROANILINE

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16. ABSTRACT

The Health and Environmental Effects Profile for 2-Methoxy-5-nitroaniline was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA) and to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents.

Quantitative estimates have been presented provided sufficient data are available. 2-Methoxy-5-nitroaniline has been evaluated as a carcinogen. The human carcinogen potency factor (q_1^*) for 2-methoxy-5-nitroaniline is 4.56×10^{-2} $(mg/kg/day)^{-1}$ for oral exposure.

The Reportable Quantity (RQ) value of 1, 10, 100, 1000 or 5000 pounds is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified by CERCLA based on chronic toxicity. The RQ value for 2-methoxy-5-nitroaniline is 1000.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field Group

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This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste and Emergency Response by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA), as well as to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched." The literature search material is current through November, 1985.

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Reference doses (RfDs) for chronic exposures. An RfD is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, RfDs are not estimated in this document series. Instead, a carcinogenic potency factor of q_1^* is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under CERCLA. These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity and acute mammalian toxicity).

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA and CERCLA dockets.

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The initial draft of this report was prepared by Syracuse Research Corporation under Contract No. 68-03-3228 for U.S. EPA's Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH. Dr. Christopher DeRosa was the Technical Project Monitor and Helen Ball was the Project Officer. The final documents in this series were prepared for the Office of Solid Waste, Washington, DC.

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EXECUTIVE SUMMARY

2-Methoxy-5-nitroaniline is an orange-red solid at ambient temperatures. It is soluble in a variety of organic solvents and in hot water (Weast, 1980; IARC, 1982). A considerable data gap exists regarding the basic physical and chemical properties of this compound. Apparently, three companies currently manufacture the chemical in the United States (USITC, 1985; SRI, 1986). Current U.S. production volume is not available. In 1983, a total of 115,400 pounds of this chemical was imported through principal U.S. customs districts (USITC, 1984). 2-Methoxy-5-nitroaniline is used almost exclusively as an intermediate in the manufacture of dyes and pigments (IARC, 1982).

Information specific to the fate and transport of 2-methoxy-5-nitroaniline in environmental media could not be located in the available literature. Based on the available physical properties and the fate and transport of structurally similar compounds, the following speculations about the fate and transport of 2-methoxy-5-nitroaniline can be made. This compound may not significantly biodegrade either in water or soil. Algae-accelerated photoreaction of this compound in water remains a possibility. The amino group in 2-methoxy-5-nitroaniline is susceptible to oxidation by RO_2 radical or other oxidants in water. In air, oxidation by OH radical may play a significant role in determining the fate of the compound. Volatilization of the compound from water or soil surfaces may not be significant. But if the compound forms a strong covalent bond with the carbonyl group of humic substances like other aromatic amines, it may remain strongly sorbed to soil and sediments containing humic materials. 2-Methoxy-5-nitroaniline may not bioconcentrate significantly in aquatic organisms.

2-Methoxy-5-nitroaniline may be found in the wastewater from dye industries using this chemical. Consumer exposure to this chemical is also possible from any residual compound present in consumer products containing this dye material. No monitoring data on the level of this compound in air, water, food or any other medium are available. The absence of data may be due to lack of adequate monitoring rather than the absence of this compound in any medium.

Pertinent data regarding effects of 2-methoxy-5-nitroaniline on aquatic organisms could not be located in the available literature as cited in the Appendix. Pertinent pharmacokinetic and metabolism studies of 2-methoxy-5-nitroaniline could not be located in the available literature as cited in the Appendix.

In the NCI (1978) study, B6C3F1 mice receiving 0.6% (TWA, dose B) and 0.8% (dose A) 2-methoxy-5-nitroaniline in the diet for 78 weeks had significantly increased incidences of liver carcinoma (dose A males, and dose B females) and leukemia/lymphoma (dose B females).

The results of mutagenicity testing of 2-methoxy-5-nitroaniline in Salmonella typhimurium were generally positive showing dose-related responses with or without S-9. 2-Methoxy-5-nitroaniline was most mutagenic in frameshift sensitive tester strains (Haworth et al., 1983). Chiu et al. (1978) reported positive findings for TA98 but not TA100 in plate incorporation assays without S-9. In Drosophila melanogaster, 2-methoxy-5-nitroaniline did not cause sex-linked recessive lethal mutations (Valencia et al., 1985). That 2-methoxy-5-nitroaniline is absorbed from the gastrointestinal tract of rats or mice can be inferred from the systemic toxicity and carcinogenicity following administration in the diet (Sections 5.1. and 5.5.). Although metabolism studies of 2-methoxy-5-nitroaniline were not

available, reduction of the nitro group to a nitroso or N-hydroxy derivative has been proposed as the mechanism of bioactivation of aromatic nitro compounds (Poirier and Weisburger, 1974; U.S. EPA, 1979).

Dietary administration of 2-methoxy-5-nitroaniline to male and female Fischer 344 rats at concentrations of 0.4 and 0.8% for 78 weeks produced neoplasms at numerous sites (NCI, 1978). Lesions that occurred with statistically significant increased incidences in rats included carcinomas of the skin (high- and low-dose males), adrenal adenomas/carcinomas (high-dose males), preputial gland adenomas/carcinomas (high-dose males), lung adenomas/carcinomas (low-dose females), clitoral gland adenomas/carcinomas (high- and low-dose females), mammary gland adenomas/carcinomas (high- and low-dose females) and Zymbal gland carcinomas (high-dose males and females).

Information regarding teratogenicity could not be located in the available literature as cited in the Appendix. Tubule degeneration in the testis of male Fischer 344 rats was found after 78 weeks of administration of 0.4 or 0.8% 2-methoxy-5-nitroaniline in the diet (NCI, 1978).

Chronic dietary administration of 2-methoxy-5-nitroaniline to Fischer 344 rats and B6C3F1 mice caused decreased body weight gain in both sexes of rats at both doses and in all groups of treated mice except dose A males, and increased mortality in male and female rats and female mice at both dose levels relative to matched controls (NCI, 1978). Nonneoplastic lesions found at increased incidences included increased hematopoiesis of the spleen in male and female rats at $\geq$0.4%; myocardial degeneration and thyroid follicle pigmentation in male and female rats at 0.8%; gastric mucosa sclerosis and liver cell degeneration in male rats at 0.8%; bone marrow myelofibrosis, kidney nephrosis and thyroid degeneration in female mice at both doses; and liver and thyroid degeneration in male mice at both doses.

A q_1^* of 4.56×10^{-2} $(mg/kg/day)^{-1}$ was derived based on the incidence of skin carcinomas in male rats in the NCI (1978) study. Water concentrations associated with increased lifetime risk levels of 10^{-5}, 10^{-6} and 10^{-7} are 8×10^{-3}, 8×10^{-4} and 8×10^{-5} mg/ℓ, respectively. An F factor of 3.36×10^{-1} $(mg/kg/day)^{-1}$ was also derived based on the incidence of skin tumors in the NCI (1978) study, placing 2-methoxy-5-nitroaniline in Potency Group 3. Because rats also had increased incidences of several unusual tumor types (Zymbal's gland carcinoma, preputial gland adenoma/carcinoma, clitoral gland adenoma/carcinoma); because NCI (1978) concluded that 2-methoxy-5-nitroaniline induced hepatocellular carcinomas in female mice; and because mutagenicity testing generally has been positive, the evidence for a carcinogenic effect in animals was considered to be sufficient. Since no data regarding the carcinogenicity to humans were located, 2-methoxy-5-nitroaniline is classified as a U.S. EPA Group B2 Chemical. Potency Group 3 and U.S. EPA Group B2 chemicals have a low hazard ranking under CERCLA. An RQ of 1000 was derived based on the increased mortality of rats in the NCI (1978) study.

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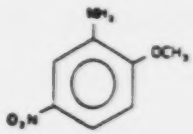
LIST OF ABBREVIATIONS

BCF	Bioconcentration factor
bw	Body weight
CAS	Chemical Abstract Service
CS	Composite score
K_{ow}	Octanol/water partition coefficient
LD_{50}	Dose lethal to 50% of recipients
MED	Minimum effect dose
MTD	Maximum tolerated dose
ppm	Parts per million
RQ	Reportable quantity
RV_d	Dose-rating value
RV_e	Effect-rating value
TWA	Time-weighted average
UV	Ultraviolet

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

2-Methoxy-5-nitroaniline is also knwon as benzenamine, 2-methoxy-5-nitro-; 5-nitro-o-anizidine; 2-amino-4-nitroanisole; o-anisidine nitrate; Azoic Diazo Component 13, base; and 5-nitro-2-methoxyaniline (U.S. EPA 1986a;). The structure, empirical formula, molecular weight and CAS Registry number for this chemical are as follows:



Empirical formula: $C_7H_8N_2O_3$

Molecular weight: 168.16

CAS Registry number: 99-59-2

1.2. PHYSICAL AND CHEMICAL PROPERTIES

2-Methoxy-5-nitroaniline is an orange-red solid at ambient temperatures. It is soluble in ethanol, acetone and acetic acid, and also in hot diethyl ether, hot benzene and hot water (IARC, 1982; Weast, 1980). Selected physical properties of this chemical are listed below:

Melting point:	118°C	Weast, 1980
Density at 156°C:	1.2068	Weast, 1980
UV absorption: maximum	374, 303, 257 and 227 nm	U.S. EPA, 1976

Other common physical properties, including vapor pressure and K_{ow}, are not available. Chemically, the nitro group of this compound is susceptible to reduction to the amino group by zinc and acid. The amino group of 2-methoxy-5-nitroaniline can form diazonium salt in the presence of HNO_2. This reaction is important in the commercial preparation of dyes (U.S. EPA, 1976).

1.3. PRODUCTION DATA

According to U.S. EPA (1977), two companies manufactured this chemical in the United States in 1977, producing volumes of <1000 and 10,000-100,000 pounds. Two other U.S. companies had production capability, but did not produce this chemical in 1977. SRI (1986) lists American Cyanamid Co., Marietta, OH, as the sole U.S. producer of this chemical as of January, 1986. USITC (1985), however, lists Alliance Chemical Inc., Newark, NJ, and Blackman Uhler Chemical Division of Synallory Corp., Augusta, GA, as the current U.S. producers of this chemical. Current U.S. production volumes are not available. In 1983, 34,016 pounds of 2-methoxy-5-nitroaniline and 81,379 pounds of the same compound under the name Azoic Diazo component 13, base, were imported through principal U.S. custom districts (USITC, 1984).

2-Methoxy-5-nitroaniline is produced either by partial reduction of 2,4-dinitroanisole or nitration of o-anisidine (Catino and Farris, 1978). In the latter process o-anisidine is nitrated with HNO_3 and H_2SO_4, followed by heating at 60°C. The mixture is then filtered and neutralized with ammonia to produce 2-methoxy-5-nitroaniline with a 69% yield (Spiess, 1974). A commercial product imported into the United States showed ~97.5% purity (Venturella et al., 1973).

1.4. USE DATA

2-Methoxy-5-nitroaniline is used almost exclusively as an intermediate in the manufacture of dyes and pigments (IARC, 1982).

1.5. SUMMARY

2-Methoxy-5-nitroaniline is an orange-red solid at ambient temperatures. It is soluble in a variety of organic solvents and in hot water (Weast, 1980; IARC, 1982). A considerable data gap exists regarding the basic physical and chemical properties of this compound. Apparently, three

companies currently manufacture the chemical in the United States (USITC, 1985; SRI, 1986). Current U.S. production volume is not available. In 1983, a total of 115,400 pounds of this chemical was imported through principal U.S. customs districts (USITC, 1984). 2-Methoxy-5-nitroaniline is used almost exclusively as an intermediate in the manufacture of dyes and pigments (IARC, 1982).

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

2.1. AIR, WATER AND SOIL

Information specific to the fate and transport of 2-methoxy-5-nitroaniline in any environmental medium could not be located in the available literature as cited in the Appendix. The biodegradability test with soil microorganisms (Alexander and Lustigman, 1966) and activated sludge (Kawasaki, 1980) suggests that nitroaromatic compounds may not significantly biodegrade in natural environmental media. The addition of a methoxy group to the disubstituted benzene ring may not alter the biodegradation pattern significantly. Therefore, it is speculated that 2-methoxy-5-nitroaniline will not significantly biodegrade either in ambient water or soil. 2-Methoxy-5-nitroaniline has two absorption maxima in the solar radiation region (see Section 1.2.). When considered with the finding of Zepp and Schlotzhauer (1983) that photoreaction of certain aromatic amines is accelerated by specific algae found in natural water, it appears that 2-methoxy-5-nitroaniline could photodegrade; however, no study is available in the literature to demonstrate the photodegradation potential of this compound. The amino group in 2-methoxy-5-nitroaniline is susceptible to oxidation. Oxidation of this compound by RO_2 radical or other oxidants present in water is likely. In air, oxidation by OH radical may transform or degrade this compound. The rate constant for OH radical reaction with 2-methoxy-5-nitroaniline is not available. Based on the structure-activity relationship by the FAP Data Base (U.S. EPA, 1986b), the rate constant for this reaction is $\sim 1.7 \times 10^{-11}$ cm^3 $molecule^{-1}$ sec^{-1}. If the concentration of OH radical in the atmosphere is assumed to be 10^6 molecules cm^{-3} (U.S. EPA 1980a), the half-life for this reaction is ~11 hours. Therefore, this reaction may play a significant role in determining the fate of 2-methoxy-5-nitroaniline in the atmosphere.

Since 2-methoxy-5-nitroaniline is soluble in hot water (see Section 1.2.), its solubility in water at ambient temperatures is expected to be significant. The vapor pressure data for this compound is not available. But the vapor pressures of the three isomers of nitroaniline are between 1.19 and 1.79 mm Hg (U.S. EPA 1976). Therefore, the vapor pressure of 2-methoxy-5-nitroaniline is expected to be even lower. Compounds with vapor pressure of <1 mm Hg and significant water solubility are not expected to volatilize significantly from water or soil surfaces. Although the soil/sediment sorption coefficient for 2-methoxy-5-nitroaniline is not available, the significant water solubility of this compound indicates that the K_{ow} of this compound will be low. Consequently, the compound is not expected to adsorb strongly to soil or sediment. Aromatic amines, however, form strong covalent bonds with humates present in soil and sediment (Parris, 1980); therefore, significant sorption may occur. No experimental or estimated data for the BCF of this compound are available in the literature as cited in the Appendix. Based on its significant water solubility and expected low K_{ow}, this compound is not likely to bioconcentrate significantly in aquatic organisms.

2.2. SUMMARY

Information specific to the fate and transport of 2-methoxy-5-nitroaniline in environmental media are not available in the literature. Based on the available physical properties and the fate and transport of structurally similar compounds, the following speculations about the fate and transport of 2-methoxy-5-nitroaniline can be made. This compound may not significantly biodegrade either in water or soil. Algae-accelerated photoreaction of this compound in water remains a possibility. The amino group in 2-methoxy-5-nitroaniline is susceptible to oxidation by RO_2 radical or

other oxidants in water. In air, oxidation by OH radical may play a significant role in determining the fate of the compound. Volatilization of the compound from water or soil surfaces may not be significant. But if the compound forms a strong covalent bond with the carbonyl group of humic substances like other aromatic amines, it may remain strongly sorbed to soil and sediments containing humic materials. 2-Methoxy-5-nitroaniline may not bioconcentrate significantly in aquatic organisms.

3. EXPOSURE

2-Methoxy-5-nitroaniline may be found in the wastewater from dye industries using this chemical. Consumer exposure to this chemical is also possible from any residual compound present in consumer products containing this dye material. No monitoring data on the level of this compound in air, water, food or any other medium are available. The absence of data may be due to lack of adequate monitoring rather than the absence of this compound in any medium.

4. PHARMACOKINETICS

Pertinent pharmacokinetic and metabolism studies of 2-methoxy-5-nitroaniline could not be located in the available literature as cited in the Appendix. That 2-methoxy-5-nitroaniline is absorbed from the gastrointestinal tract of rats of mice can be inferred from the systemic toxicity and carcinogenicity following administration in the diet (Sections 5.1. and 5.5.). Although metabolism studies of 2-methoxy-5-nitroaniline were not available, reduction of the nitro group to a nitroso or N-hydroxy derivative has been proposed as the mechanism of bioactivation of aromatic nitro compounds (Poirier and Weisburger, 1974; U.S. EPA, 1979).

5. EFFECTS

5.1. CARCINOGENICITY

In an NCI (1978) bioassay, groups of 50 male and 50 female Fischer 344 rats were fed diets containing 0.4 or 0.8% 2-methoxy-5-nitroaniline for 78 weeks and observed for an additional 24-28 weeks. Separate control groups of 50 male and 50 female rats/dose level were supplied untreated feed for 108-109 weeks. The low-dose rats were obtained from a different supplier than the other groups. Survival after 85 weeks for the females was 58% for high-dose, 74% for low-dose, 86% for high-dose controls and 88% for low-dose controls. Mortality increased sharply in the males after 71 weeks, with survival at 85 weeks ~28% for high-dose, 50% for low-dose and 95% for both control groups. The Cox tests indicated there was a significantly greater mortality in both sexes of the high- and the low-dose groups than their respective control groups; however, adequate numbers of rats survived to be at risk from late-developing tumors. Comprehensive histological examinations were performed on each rat that died, was killed when moribund or was sacrificed at the end of the study.

As shown in Table 5-1, there was a statistically significant increase (Fisher Exact test) in the incidence of skin carcinomas in males of both dose groups relative to their respective controls. The incidences of adrenal and preputial gland adenomas/carcinomas were significantly increased in high-dose males compared with controls. In the females, lung adenomas/carcinomas showed a statistically significant elevation in the low-dose group, but not in the high-dose group, relative to their respective controls. Clitoral gland carcinomas were significantly increased in high-dose females. Statistical significance was achieved in both dose groups, as compared with matched controls, when clitoral gland adenomas and carcinomas

TABLE 5-1

Incidence of Tumors in Rats and Mice Treated with 2-Methoxy-5-nitroaniline in the Diet for 78 Weeks[a,b]

Species/ Strain	Sex	Concentration in Diet (%)	Duration of Study (weeks)	Target Organ	Tumor Type	Tumor Incidence[c] (p value)
Rat/F344	M	0 (low dose control)	108	skin	all carcinomas[d]	1/48
				adrenal	adenoma/carcinoma	1/47
				preputial gland	adenoma/carcinoma	2/48
				Zymbal's gland[e]	all carcinomas[f]	1/48
	M	0.4	106	skin	all carcinomas[d]	30/50 (p<0.001)
				adrenal	adenoma/carcinoma	1/48
				preputial gland	adenoma/carcinoma	2/50
				Zymbal's gland[e]	all carcinomas[f]	2/50
	M	0 (high dose control)	109	skin	all carcinomas[d]	0/48
				adrenal	adenoma/carcinoma	0/47
				preputial gland	adenoma/carcinoma	0/48
				Zymbal's gland[e]	all carcinomas[f]	0/48
	M	0.8	102	skin	all carcinomas[d]	40/48 (p<0.001)
				adrenal	adenoma/carcinoma	5/48 (p=0.030)
				preputial gland	adenoma/carcinoma	5/48 (p=0.028)
				Zymbal's gland[e]	all carcinomas[f]	10/48 (p=0.001)
	F	0 (low dose control)	108	lung	adenoma/carcinoma	0/49
				clitoral gland	carcinoma	0/49
				clitoral gland	adenoma/carcinoma	1/49
				Zymbal's gland[e]	carcinoma[f]	0/49
				mammary gland	carcinoma	0/49
				mammary gland	adenoma/carcinoma	2/49
	F	0.4	106	lung	adenoma/carcinoma	5/49 (p=0.028)
				clitoral gland	carcinoma	1/49
				clitoral gland	adenoma/carcinoma	12/49 (p=0.001)
				Zymbal's gland[e]	carcinomas[f]	3/49
				mammary gland	carcinoma	10/49 (p=0.001)
				mammary gland	adenoma/carcinoma	11/49 (p=0.007)
	F	0 (high dose control)	109	lung	adenoma/carcinoma	1/50
				clitoral gland	carcinoma	0/50
				clitoral gland	adenoma/carcinoma	2/50
				Zymbal's gland[e]	carcinomas[f]	0/50
				mammary gland	carcinoma	0/50
				mammary gland	adenoma/carcinoma	0/50

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TABLE 5-1 (cont.)

Species/ Strain	Sex	Concentration in Diet (%)	Duration of Study (weeks)	Target Organ	Tumor Type	Tumor Incidence[c] (p value)
Rat/F344	F	0.8	106	lung	adenoma/carcinoma	1/43
				clitoral gland	carcinoma	9/46 (p=0.001)
				clitoral gland	adenoma/carcinoma	14/46 (p<0.001)
				Zymbal's gland[e]	carcinoma[f]	7/46 (p=0.004)
				mammary gland	carcinoma	4/46 (p=0.049)
				mammary gland	adenoma/carcinoma	4/46 (p=0.049)
Mouse/B6C3F1	M	0 (dose B control)	96	liver	hepatocellular carcinoma	6/48
		0.6 (dose B)[g]	96	liver	hepatocellular carcinoma	3/47
		0 (dose A control)	95	liver	hepatocellular carcinoma	12/50
		0.8 (dose A)	96	liver	hepatocellular carcinoma	25/48 (p=0.004)
	F	0 (dose B control)	96	liver	hepatocellular carcinoma	1/50
				hematopoietic system	leukemia/lymphoma	2/50
		0.6 (dose B)[g]	96	liver	hepatocellular carcinoma	8/43 (p=0.008)
				hematopoietic system	leukemia/lymphoma	10/45 (p=0.008)
		0 (dose A control)	96	liver	hepatocellular carcinoma	2/47
				hematopoietic system	leukemia/lymphoma	7/48

TABLE 5-1 (cont.)

Species/ Strain	Sex	Concentration in Diet (%)	Duration of Study (weeks)	Target Organ	Tumor Type	Tumor Incidence[c] (p value)
Mouse/B6C3F1	F	0.8 (dose A)	97	liver	hepatocellular carcinoma	0/41
				hematopoietic system	leukemia/lymphoma	5/43

QUALITY OF EVIDENCE

Strengths of Study: The compound was administered for the majority of the animals' lifespan by a relevant route at two dose levels in both sexes of two species. Sufficient numbers of animals survived for analysis of late-developing tumors. Comprehensive histological examinations and appropriate statistics were done. The MTD, determined from preliminary subchronic studies, was used.

Weakness of Study: One treatment group in each species was obtained from a different supplier than the rest of the groups. The dose schedule changed in one species.

Overall Adequacy: Adequate

[a]Source: NCI, 1978

[b]The compound was analyzed and found to be of high purity.

[c]Number of animals with tumors/number of animals examined microscopically for the organ

[d]Squamous-cell carcinoma, basal-cell carcinoma, trichoepithelioma, sebaceous adenocarcinoma, sweat-gland carcinoma, excludes tumors on the skin of the ear.

[e]Includes skin of ear

[f]Sebaceous adenocarcinoma, ceruminous carcinoma, squamous cell carcinoma

[g]TWA

were combined. The incidences of mammary carcinoma and mammary adenoma/carcinoma were also significantly elevated in both dose groups. In both the male and female high-dose groups, Zymbal's gland carcinomas were significantly increased.

Groups of 50 male and 50 female B6C3F1 mice were treated with 0.8% (dose A) 2-methoxy-5-nitroaniline in the diet for 78 weeks and observed for an additional 18 weeks. Another group of 50 mice of both sexes was fed a diet containing 1.6% of the compound for 15 weeks, after which the concentration was lowered to 0.4% for 63 weeks (TWA for 78 weeks = 0.6%, dose B). The designations dose A and B were used instead of high and low dose because the group that originally received the higher dose ultimately received a lower TWA dose. Separate controls for dose A and dose B groups were fed untreated diets for 95-96 weeks. Dose A mice were obtained from a different supplier than the other groups. Survival of the males at the end of the study was 88% for dose B, 84% for dose A, 80% for dose B controls and 86% for dose A controls and survival for females was 66% for dose B, 32% for dose A, 76% for dose B controls and 72% for dose A controls. The greater mortality in dose A and B females was significantly greater than the respective control group (Cox Test).

The incidence of hepatocellular carcinomas was significantly increased in dose A males relative to dose A controls. In the females, dose B mice had significantly elevated incidences of both hepatocellular carcinoma and leukemia/lymphoma compared with the matched control group. Because the dose A males were from a different supplier and the incidence of leukemia/lymphoma in the dose B females was comparable with dose A controls, only the increased incidence of hepatocellular carcinoma in the females was considered to be compound-related (NCI, 1978).

NCI (1978) concluded that administration of 2-methoxy-5-nitroaniline in the diet induced carcinomas of the Zymbal gland in both sexes of rats, carcinomas of the skin in male rats and clitoral gland neoplasms in female rats. The compound also increased the incidence of hepatocellular carcinomas in female mice.

5.2. MUTAGENICITY

Results of mutagenicity assays of 2-methoxy-5-nitroaniline are summarized in Table 5-2. Positive results for reverse mutation were obtained in mutagenicity tests with Salmonella typhimurium TA98 both with and without S-9 (Chiu et al., 1978; Haworth et al., 1983). 2-Methoxy-5-nitroaniline did not produce a clear dose-related response in S. typhimurium strain TA100 when tested by plate incorporation without S-9 by Chiu et al. (1978). Haworth et al. (1983), however, found an increase in the number of revertants in strains TA98, TA100 (frameshift sensitive) and TA1537 (base pair substitution sensitive), but no effect in strain TA1535, both with and without S-9 metabolic activation when tested using a pre-incubation procedure. Although a dose-related response was obtained in TA1535, it was much less sensitive than those observed in TA98 and TA100.

There were no increases in sex-linked recessive lethal mutations in Drosophila melanogaster treated with 500 and 10,000 ppm 2-methoxy-5-nitroaniline in the diet or by injection (Valencia et al., 1985). Equivocal results were reported in an injection experiment at 500 ppm.

5.3. TERATOGENICITY

Pertinent data regarding the teratogenicity of 2-methoxy-5-nitroaniline could not be located in the available literature as cited in the Appendix.

TABLE 5-2

Mutagenicity Testing of 2-Methoxy-5-nitroaniline

Assay	Indicator Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comments	Reference
Reverse mutation	Salmonella typhimurium TA98 TA100	NR	plate incorporation	0.1-10 µmol/ plate	none added	+ -	NC	Chiu et al., 1978
	S. typhimurium TA98 TA100 TA1535 TA1537	99.9%	plate incorporation	10-2000 µg/plate	±S-9	+ + + +	Strains were preincubated with 2-methoxy-5-nitroaniline before plating	Haworth et al., 1983
Sex-linked recessive lethal	Drosophila melanogaster	99.9%	diet	500 or 10,000 ppm	NA	-	Negative result prompted testing by injection	Valencia et al., 1985
			injection	500 or 10,000 ppm	NA	?	Equivocal results reported for the 500 ppm dose	Valencia et al., 1985

NA = Not applicable; NC = no comment; NR = not reported

5.4. OTHER REPRODUCTIVE EFFECTS

A dose-related statistically significant increased incidence of tubule degeneration in the testis was found in male Fischer 344 rats administered 0.4 or 0.8% 2-methoxy-5-nitroaniline in the diet for 78 weeks (NCI, 1978) (Section 5.5.).

5.5. SUBCHRONIC AND CHRONIC TOXICITY

In order to establish MTDs for the carcinogenicity studies (see Section 5.1.), NCI (1978) conducted subchronic toxicity tests. 2-Methoxy-5-nitroaniline was administered for 7 weeks in the diets to groups of five male and five female Fischer 344 rats and five male and five female B6C3F1 mice at concentrations of 0, 0.05, 0.1, 0.2 or 0.4%. There was no mortality in either species. A dietary concentration of 0.4% produced decreases in body weight of 10% in female rats and 7.9% in male mice. The body weights of high-dose male rats and female mice were not depressed. The only compound-related abnormalities observed were darkened spleens in two female rats receiving 0.4% 2-methoxy-5-nitroaniline in the diet.

The chronic toxicity data in the NCI (1978) bioassay showed a dose-related decrease (compared with controls) in mean body weight gain in rats of both sexes throughout the 78 weeks of treatment and during the 24- to 28-week observation period. The decreases were significant for each dosed group compared with its respective control. Dose-related mortality of both dose groups was significantly greater and occurred earlier than in the controls. The incidences of significantly increased nonneoplastic lesions are presented in Table 5-3. Nonneoplastic lesions were associated with testicular and hepatocellular degeneration and gastric mucosal sclerosis in male rats. Treated rats of both sexes had increased incidences of pigmentation of thyroid follicles, increased spleen hematopoiesis and myocardial degeneration relative to the controls.

TABLE 5-3

Incidence of Significantly Increased Nonneoplastic Lesions in Fischer 344 Rats Treated with 2-Methoxy-5-nitroaniline in the Diet for 78 Weeks[a,b,c]

Tissue, Type of Lesion	Incidence			
	0 (Low dose controls)	0 (high dose controls)	0.4%	0.8%
Males				
Spleen, lymphoid depletion	0/48	0/48	0/47	6/48 (p=0.013)
Spleen, increased hematopoiesis	0/48	0/48	4/47 (p=0.056)	16/48 (p<0.001)
Myocardium, degeneration	1/48	0/48	2/49	15/48 (p<0.001)
Liver, hepatocyte degeneration "ballooning"	0/48	0/48	0/48	14/48 (p<0.001)
Gastric mucosa, sclerosis	0/48	0/48	0/46	10/48 (p<0.001)
Thyroid, follicle pigmentation	0/39	0/48	0/47	39/47 (p<0.001)
Testis, tubule degeneration	0/47	0/47	8/48 (p=0.003)	34/48 (p<0.001)
Females				
Spleen, increased hematopoiesis	0/48	0/48	12/48 (p<0.001)	13/43 (p<0.001)
Myocardium, degeneration	0/49	0/50	2/48	7/43 (p=0.003)
Thyroid, follicle pigmentation	0/40	0/45	0/49	5/42 (p<0.023)

[a]Source: NCI, 1978

[b]Incidence expressed as number rats with lesions/number of rats examined microscopically at that organ or tissue

[c]Fisher Exact test performed at SRC

A moderate decrease in mean body weight gain was found in all treated mice, except dose A (0.8%) males, relative to controls. No statistically significant difference in mortality was found between treated and control males; however, mortality was significantly greater in both treated groups of female mice compared with matched control groups. Nonneoplastic lesions with statistically significantly increased incidences are presented in Table 5-4. These included nephrosis, thyroid degeneration and bone marrow myelofibrosis in females and thyroid and hepatocellular degeneration, and hepatocellular hyperplasia in males.

5.6. OTHER RELEVANT INFORMATION

The oral LD_{50} of 2-methoxy-5-nitroaniline for rats was reported to be 704 mg/kg and for mice was 1060 mg/kg (NIOSH, 1986). Schafer et al. (1985) estimated an oral LD_{50} for deer mice, Peromyscus maniculatus, of >1050 mg/kg based on consumption for 3 days of Douglas fir seeds treated with 2-methoxy-5-nitroaniline.

5.7. SUMMARY

Dietary administration of 2-methoxy-5-nitroaniline to male and female Fischer 344 rats at concentrations of 0.4 and 0.8% for 78 weeks produced neoplasms at numerous sites (NCI, 1978). Lesions that occurred with statistically significant increased incidences in rats included carcinomas of the skin (high- and low-dose males), adrenal adenomas/carcinomas (high-dose males), preputial gland adenomas/carcinomas (high-dose males), lung adenomas/carcinomas (low-dose females), clitoral gland adenomas/carcinomas (high- and low-dose females), mammary gland adenomas/carcinomas (high- and low-dose females) and Zymbal gland carcinomas (high-dose males and females).

TABLE 5-4

Incidences of Significantly Increased Nonneoplastic Lesions in B6C3F1 Mice Treated with 2-Methoxy-5-nitroaniline in the Diet for 78 Weeks[a,b,c]

Tissue, Type of Lesion	Incidence			
	0 (Dose B control)	0 (Dose A control)	0.6% (Dose B)	0.8% (Dose A)
Males				
Liver, hyperplasia	1/50	0/48	0/48	21/47 (p<0.001)
Liver, angiectasis	1/50	0/48	19/48 (p<0.001)	19/47 (p<0.001)
Liver, hepatocyte degeneration	0/50	0/48	20/48 (p<0.001)	22/47 (p<0.001)
Thyroid, degeneration	0/40	0/45	41/47 (p<0.001)	44/47 (p<0.001)
Females				
Bone Marrow, myelofibrosis	1/46	0/49	15/35 (p<0.001)	31/40 (p<0.001)
Kidney, hydronephrosis	0/45	0/50	4/42 (p=0.050)	25/43 (p<0.001)
Kidney, interstitial inflammation	1/45	12/50	15/42 (p<0.001)	35/43 (p<0.001)
Kidney, nephrosis	0/45	0/50	15/42 (p<0.001)	32/43 (p<0.001)
Thyroid, hyperpigmentation	0/41	0/44	11/34 (p<0.001)	0/43
Thyroid, degeneration	0/41	0/44	12/34 (p<0.001)	8/43 (p=0.002)

[a]Source: NCI, 1978

[b]Incidence expressed as number of mice with lesions/number of mice examined microscopically at that organ or tissue

[c]Fisher Exact test performed at SRC

In the same NCI (1978) study, B6C3F1 mice receiving 0.6% (TWA, dose B) and 0.8% (dose A) 2-methoxy-5-nitroaniline in the diet for 78 weeks had significantly increased incidences of liver carcinoma (dose A males, and dose B females) and leukemia/lymphoma (dose B females).

The results of mutagenicity testing of 2-methoxy-5-nitroaniline in Salmonella typhimurium were generally positive. Reverse mutations were induced in strains TA98, TA100 and TA1537, but not TA1535, both with and without S-9 (Haworth et al., 1983). Chiu et al. (1978) reported positive findings for TA98 but not TA100 in plate incorporation assays without S-9. In Drosophila melanogaster, 2-methoxy-5-nitroaniline did not cause sex-linked recessive lethal mutations (Valencia et al., 1985).

Information regarding teratogenicity was not located in the available literature as cited in the Appendix. Tubule degeneration in the testis of male Fischer 344 rats was found after 78 weeks of administration of 0.4 or 0.8% 2-methoxy-5-nitroaniline in the diet (NCI, 1978).

Chronic dietary administration of 2-methoxy-5-nitroaniline to Fischer 344 rats and B6C3F1 mice caused decreased body weight gain in both sexes of rats at both doses and in all groups of treated mice except dose A males, and increased mortality in male and female rats and female mice at both dose levels relative to matched controls (NCI, 1978). Nonneoplastic lesions found at increased incidences included increased hematopoiesis of the spleen in male and female rats at $\geq$0.4%, myocardial degeneration and thyroid follicle pigmentation in male and female rats at 0.8%, gastric mucosa sclerosis and liver cell degeneration in male rats at 0.8%, bone marrow myelofibrosis, kidney nephrosis and thyroid degeneration in female mice at both doses, and liver and thyroid degeneration in male mice at both doses.

6. AQUATIC TOXICITY

Pertinent data regarding effects of 2-methoxy-5-nitroaniline on aquatic organisms could not be located in the available literature as cited in the Appendix.

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

Pertinent guidelines and standards, including EPA ambient water and air quality criteria, drinking water standards, FAO/WHO ADIs, EPA or FDA tolerances for raw agricultural commodities or foods, and ACGIH, NIOSH or OSHA occupational exposure limits could not be located in the available literature as cited in the Appendix.

7.2. AQUATIC

Guidelines and standards for the protection of aquatic biota from the effects of 2-methoxy-5-nitroaniline could not be located in the available literature as cited in the Appendix.

8. RISK ASSESSMENT

Dietary administration of 2-methoxy-5-nitroaniline to male and female Fischer 344 rats at concentrations of 0.4 and 0.8% for 78 weeks produced neoplasms at numerous sites (NCI, 1978). Lesions that occurred with statistically significant increased incidences in rats included carcinomas of the skin (high- and low-dose males), adrenal adenomas/carcinomas (high-dose males), preputial gland adenomas/carcinomas (high-dose males), lung adenomas/carcinomas (low-dose females), clitoral gland adenomas/carcinomas (high- and low-dose females), mammary gland adenomas/carcinomas (high- and low-dose females) and Zymbal gland carcinomas (high-dose males and females).

In the same NCI (1978) study, B6C3F1 mice receiving 0.6% (TWA, dose B) and 0.8% (dose A) 2-methoxy-5-nitroaniline in the diet for 78 weeks had significantly increased incidences of liver carcinoma (dose A males, and dose B females), and leukemia/lymphoma (dose B females).

The results of mutagenicity testing of 2-methoxy-5-nitroaniline in Salmonella typhimurium were generally positive. Reverse mutations were induced in strains TA98, TA100 and TA1537, but not TA1535, both with and without S-9 (Haworth et al., 1983). Chiu et al. (1978) reported positive findings for TA98 but not TA100 in plate incorporation assays without S-9. In Drosophila melanogaster, 2-methoxy-5-nitroaniline did not cause sex-linked recessive lethal mutations (Valencia et al., 1985).

Information regarding teratogenicity could not be located in the available literature as cited in the Appendix. Tubule degeneration in the testis of male Fischer 344 rats was found after 78 weeks of administration of 0.4 or 0.8% 2-methoxy-5-nitroaniline in the diet (NCI, 1978).

Chronic dietary administration of 2-methoxy-5-nitroaniline to Fischer 344 rats and B6C3F1 mice caused decreased body weight gain in both sexes of rats at both doses and in all groups of treated mice except dose A males, and increased mortality in male and female rats and female mice at both dose levels relative to matched controls (NCI, 1978). Nonneoplastic lesions found at increased incidences included increased hematopoiesis of the spleen in male and female rats at $\geq$0.4%, myocardial degeneration and thyroid follicle pigmentation in male and female rats at 0.8%, gastric mucosa sclerosis and liver cell degeneration in male rats at 0.8%, bone marrow myelofibrosis, kidney nephrosis and thyroid degeneration in female mice at both doses, and liver and thyroid degeneration in male mice at both doses.

Since chronic dietary administration of 2-methoxy-5-nitroaniline is carcinogenic to both rats and mice (NCI, 1978), it is appropriate to derive a q_1^*. Male rats exposed to 0, 0.4 and 0.8% 2-methoxy-5-nitroaniline in the diet for 78 weeks developed neoplasms at several sites. These dietary levels correspond to average intakes of 0, 200 and 400 mg/kg/day, using standard food consumption estimates for rats (0.05 kg food/kg bw/day). Data used to derive the q_1^* are presented in Table 8-1. Because NCI (1978) did not provide individual animal pathology data, it is not possible to pool incidences of all statistically significant tumor types; therefore, the incidence of skin carcinomas in the male rats is used as the basis of the q_1^*.

The length of the exposure (le) was less than the length of the experiment (Le); therefore, the doses were corrected by the ratio of le/Le, where Le (104 weeks) was defined as the mean length of the experiment for the two treatment groups (102 and 106 weeks; see Table 5-1). No adjustment for lifespan of the animal was made since this was equivalent to the length of

TABLE 8-1

Derivation of q_1^* for 2-Methoxy-5-nitroaniline

Reference: NCI, 1978

Species, strain, sex: rat, F344, male

Route, vehicle: oral, diet

Length of exposure (le) = 78 weeks

Length of experiment (LE) = 104 weeks

Lifespan of animal (L) = 104 weeks

Body weight (measured) = 0.4 kg (controls); 0.325 kg (low dose); 0.275 kg (high dose)

Tumor site and type: skin, carcinoma

Exposure	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested
0 (low dose control)	0	1/48
0.4%	25.0	30/50
0 (high dose control)	0	0/48
0.8%	47.3	40/48

Unadjusted q_1^* = not calculated (see text)

Human $q_1^* = 4.56 \times 10^{-2}$ $(mg/kg/day)^{-1}$

the experiment. Because the weights of the male rats in different treatment groups varied, each dose was transformed to the corresponding human dose before calculating the q_1^* by multiplying the rat dose by the cube root of the ratio of the average rat body weight (estimated from growth curves in the study) to the reference human body weight (70 kg) (U.S. EPA, 1980b). From these data (see Table 8-1), a human q_1^* of 4.56×10^{-2} $(mg/kg/day)^{-1}$ was calculated using the computerized multistage model by Howe and Crump (1982). Values for q_1^* based on the incidence of other tumors in male rats were also calculated (Tables 8-2, 8-3 and 8-4) and are included in order to show the contribution of these tumors to the overall risk. Although values for q_1^* could also be calculated for tumors in the female rats or in the mice, they would be lower than the q_1^* based on skin carcinomas in male rats.

As seen from Tables 8-1 to 8-4, the data that result in the highest q_1^* of 4.56×10^{-2} $(mg/kg/day)^{-1}$ are for the incidence of skin carcinomas in male rats. This q_1^* is recommended for estimating the increased lifetime risk of cancer for human exposure to 2-methoxy-5-nitroaniline. The water concentrations associated with risk levels of 10^{-5}, 10^{-6} and 10^{-7} are 8×10^{-3}, 8×10^{-4} and 8×10^{-5} mg/ℓ, respectively.

As mentioned in Chapter 5, the low-dose rats were obtained from a different supplier. An analysis was conducted to determine whether this difference between low-dose and high-dose rats, as well as other differences in body weight and experiment duration, substantially affect the potency estimate. The potency using only the low-dose rats and their controls is 4.9×10^{-2} mg/kg/day; using only the high-dose rats and their controls it is 5.1×10^{-2} mg/kg/day. Therefore, these factors do not substantially affect the potency estimate.

TABLE 8-2

Derivation of q_1^* for 2-Methoxy-5-nitroaniline

Reference: NCI, 1978

Species, strain, sex: rat, F344, male

Body weight (measured) = 0.4 kg (controls); 0.325 kg (low dose); 0.275 kg (high dose)

Route, vehicle: oral, diet

Length of exposure (le) = 78 weeks

Length of experiment (LE) = 104 weeks

Lifespan of animal (L) = 104 weeks

Tumor site and type: Zymbal's gland, carcinomas

Exposure	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested
0 (low dose control)	0	1/48
0.4%	25.0	2/50
0 (high dose control)	0	0/48
0.8%	47.3	10/48

Unadjusted q_1^* = not calculated (see text)

Human $q_1^* = 3.76 \times 10^{-3}$ $(mg/kg/day)^{-1}$

TABLE 8-3

Derivation of q_1^* for 2-Methoxy-5-nitroaniline

Reference: NCI, 1978

Species, strain, sex: rat, F344, male

Body weight (measured) = 0.4 kg (controls); 0.325 kg (low dose); 0.275 kg (high dose)

Route, vehicle: oral, diet

Length of exposure (le) = 78 weeks

Length of experiment (LE) = 104 weeks

Lifespan of animal (L) = 104 weeks

Tumor site and type: preputial gland, adenoma/carcinomas

Exposure	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested
0 (low dose control)	0	2/48
0.4%	25.0	2/50
0 (high dose control)	0	0/48
0.8%	47.3	5/48

Unadjusted q_1^* = not calculated (see text)

Human $q_1^* = 3.05 \times 10^{-3}$ $(mg/kg/day)^{-1}$

TABLE 8-4

Derivation of q_1^* for 2-Methoxy-5-nitroaniline

Reference: NCI, 1978

Species, Strain, sex: rat, F344, male

Body weight (measured) = 0.4 kg (controls); 0.325 kg (low dose); 0.275 kg (high dose)

Route, vehicle: oral, diet

Length of exposure (le) = 78 weeks

Length of experiment (LE) = 104 weeks

Lifespan of animal (L) = 104 weeks

Tumor site and type: adrenal, adenoma/carcinoma

Exposure	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested
0 (low dose control)	0	1/47
0.4%	25.0	1/48
0 (high dose control)	0	0/47
0.8%	47.3	5/48

Unadjusted q_1^* = not calculated (see text)

Human q_1^* = 2.62×10^{-3} $(mg/kg/day)^{-1}$

9. REPORTABLE QUANTITIES

9.1. REPORTABLE QUANTITIES (RQ) RANKING BASED ON CHRONIC TOXICITY

Information regarding nonneoplastic effects of chronic exposure to 2-methoxy-5-nitroaniline was provided in an NCI (1978) cancer bioassay. The toxic effects in this study are discussed in Section 5.5. and are summarized in Table 9-1.

Transformed doses were calculated by multiplying the dietary concentrations by food factors of 0.05 for rats and 0.13 for mice. These factors are based on the assumption that rats and mice consume a daily amount of food equal to 5 and 13%, respectively, of their body weight. Equivalent human doses were calculated by multiplying the transformed animal dose by the cube root of the ratio of the animal body weight to the reference human weight of 70 kg. The average body weights of the various groups of rats and mice were estimated from growth curves provided by NCI (1978).

As seen from Table 9-1 the most serious effect of 2-methoxy-5-nitroaniline is increased mortality. Because many of the animals that died or were sacrificed when found moribund during the study had tumors, it is possible that the increased mortality was secondary to the carcinogenic effects (see Section 5.1.) of the compound. For the rats, the most prevalent tumors were carcinomas of the skin; however, these carcinomas probably were not life-threatening unless they had metastasized. NCI (1978) reported low incidences of secondary tumors. Therefore, it is appropriate to consider the increased mortality as an effect of chronic exposure to 2-methoxy-5-nitroaniline that is unrelated to the carcinogenicity.

TABLE 9-1

Oral Toxicity Summary for 2-Methoxy-5-nitroaniline Administered in Diet*

Species/ Strain	Sex	No. at Start	Average Weight	Dose/ Exposure	Transformed Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Response
Rat/F344	M	50/group	0.325 kg, low-dose group; 0.275 kg, high-dose group	0.4, 0.8% in diet for 78 weeks	200, 400	33.4, 63.1	Increased mortality, testicular tubule degeneration, spleen hematopoiesis, decreased body weight gain at ≥200 mg/kg/day; hepatocyte degeneration, (ballooning), gastric mucosal sclerosis, thyroid follicle pigmentation, myocardial degeneration at 400 mg/kg/day
Rat/F344	F	50/group	0.190 kg, low-dose group; 0.175 kg, high-dose group	0.4, 0.8% in diet for 78 weeks	200, 400	27.9, 54.3	Spleen hematopoiesis, decreased body weight gain at ≥200 mg/kg/day; thyroid follicle pigmentation, myocardial degeneration at 400 mg/kg/day; increased mortality at ≥200 mg/kg/day
Mouse/ B6C3F1	M	50/group	0.035 kg	0.6, 0.8% in diet for 78 weeks	780, 1040	61.9, 82.5	Thyroid and hepatocyte degeneration, decreased body weight gain at ≥780 mg/kg/day, diffuse hepatocyte hyperplasia at 1040 mg/kg/day
Mouse/ B6C3F1	F	50/group	0.026 kg	0.6, 0.8% in diet for 78 weeks	780, 1040	56.1, 74.8	Increased mortality, bone marrow myelofibrosis, nephrosis and thyroid degeneration at ≥780 mg/kg/day

*Source: NCI, 1978

The lowest equivalent human dose at which increased mortality was observed is 27.9 mg/kg/day, the lowest dose tested. Multiplying by 70 kg results in an MED of 1953 mg/day, which corresponds to an RV_d of 1 (Table 9-2). Increased mortality warrants an RV_e of 10. Multiplying the RV_d by the RV_e results in a CS of 10, which corresponds to an RQ of 1000. The other nonneoplastic effects occurred at equivalent human doses of 27.9 mg/kg/day. Because these other effects would be assigned RV_es <10, there is no need to calculate CSs.

Therefore, the RQ of 1000 for 2-methoxy-5-nitroaniline is based on the increased mortality of rats treated with a dietary level of 0.4% for 78 weeks (Table 9-3).

9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY

The information regarding carcinogenicity of 2-methoxy-5-nitroaniline was discussed in Section 5.1. Chronic dietary exposure to 2-methoxy-5-nitroaniline resulted in significantly increased incidences of tumors at several sites in both male and female rats (NCI, 1978). The most significant finding was the dose-related increase in skin carcinoma in male rats relative to controls (see Table 5-1). Male rats at the high-dose level also had increased incidence of Zymbal's gland carcinomas, preputial gland adenoma/carcinoma and adrenal gland adenoma/carcinomas. Female rats had increased incidences of lung adenoma/carcinoma (low dose relative to controls), clitoral gland adenomas/carcinomas (low and high doses), Zymbal's gland carcinomas (high dose relative to controls) and mammary adenoma/carcinoma (high and low doses).

TABLE 9-2

Oral Composite Score for 2-Methoxy-5-nitroaniline in the Rat*

Animal Dose (mg/kg/day)	Chronic Human MED (mg/day)	RV_d	Effect	RV_e	CS	RQ
200	1953	1	increased mortality	10	10	1000

*Source: NCI, 1978

TABLE 9-3

2-Methoxy-5-nitroaniline

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral
Dose*:	1953 mg/kg
Effect:	increased mortality
Reference:	NCI, 1978
RV_d:	1
RV_e:	10
Composite Score:	10
RQ:	1000

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*Equivalent human dose

High-dose male and low-dose female mice had increased incidences of liver carcinoma relative to matched controls. Low-dose female mice also had increased incidences of leukemia/lymphoma. NCI (1978) noted, however, that the incidences of liver tumors in the treated male mice and leukemia/lymphoma in females were comparable with historic controls. The low-dose rats and 8 ppm dose mice were obtained from a different commercial supplier than the controls and other treatment groups. It cannot be ascertained, therefore, that all differences in tumor incidence between these groups and their controls are due to 2-methoxy-5-nitroaniline. Although IARC (1982) concluded that there was limited evidence for animal carcinogenicity for this compound based on the NCI (1978) study, because of the high incidences of skin carcinomas in high-dose male rats, the statistically significant increased incidences of several unusual tumor types (Zymbal's gland carcinoma, preputial gland adenoma/carcinoma, clitoral gland adenoma/carcinoma) in rats and the generally positive mutagenicity tests (see Section 5.2.), it seems more appropriate to regard the evidence for carcinogenicity in animals as sufficient. Furthermore, NCI (1978) concluded that 2-methoxy-5-nitroaniline was carcinogenic to female mice. No case reports or epidemiological studies relating 2-methoxy-5-nitroaniline to cancer in humans have been located; therefore, the evidence in humans is inadequate. 2-Methoxy-5-nitroaniline is most appropriately classified as a U.S. EPA Group B2 chemical, indicating that it is a probable human carcinogen (U.S. EPA, 1986c).

A potency (F) factor for carcinogenicity can be derived for 2-methoxy-5-nitroaniline from the data for skin carcinogenicity in male rats (NCI, 1978). The data for these calculations are presented in Table 9-4. The transformed doses [0, 0, 25.0, 47.3 mg/kg/day were calculated from dietary concentrations using a food factor of 0.05 and adjusted for differences in

TABLE 9-4

Derivation of Potency Factor (F) for 2-Methoxy-5-nitroaniline

Reference:	NCI, 1978			
Exposure route:	oral			
Species:	rat			
Strain:	F344			
Sex:	male			
Vehicle or physical state:	diet			
Body weight (measured):	0.40 kg (controls); 0.325 kg (low dose); 0.275 kg (high dose)			
Duration of treatment:	78 weeks			
Duration of study:	104 weeks			
Lifespan of animal:	104 weeks			
Target organ:	integumentary			
Tumor type:	carcinoma			
Experimental doses/exposure:	0	0	0.4%	0.8%
Transformed doses (mg/kg/day):	0	0	25.0	47.3
Tumor incidence:	1/48	0/48	30/50	40/48
Unadjusted $1/ED_{10}$:	not calculated (see text)			
Adjusted $1/ED_{10}$ (F factor):	3.36×10^{-1} $(mg/kg/day)^{-1}$			

duration of treatment (le) and duration of the study (Le) by multiplying the transformed animal dose by le/Le (78/104 weeks)]. The doses were further adjusted for differences in body weight for each treatment group by multiplying the dose by the cube root of the ratio of animal to human (70 kg) body weight. Thus, the dose was adjusted to extrapolate from rats to humans before calculation of the F factor. The F factor of 3.36×10^{-1} $(mg/kg/day)^{-1}$ was calculated using the computerized multistage model developed by Howe and Crump (1982).

Since the F factor is <1, 2-methoxy-5-nitroaniline is placed in Potency Group 4. A U.S. EPA Group B2 and a Potency Group 3 chemical has a LOW hazard corresponding to a 100 lb. RQ for potential carcinogenicity.

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APPENDIX

LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of the following:

GLOBAL
TSCATS
CASR online (U.S. EPA Chemical Activities Status Report)
CAS online STN International
TOXLINE
TOXBACK 76
TOXBACK 65
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP
NTIS
Federal Register

These searches were conducted in October, 1985. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7), and the following secondary sources were reviewed:

ACGIH (American Conference of Governmental Industrial Hygienists). 1986. Documentation of the Threshold Limit Values and Biological Exposure Indices, 5th ed. Cincinnati, OH.

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In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

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